Phytochemical and Pharmacological Evaluation of *Kigelia africana* and *Cannabis indica* for Their Aphrodisiac Potential

A Synopsis

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1. Introduction

The use of plants and plant products as medicines can be traced as far back as the beginning of human existence on this planet earth. The earliest record of medicinal plant use in the Himalayas is found in the **Rigveda**[1]. This work was written between 4500 BC and 1600 BC, is supposed to be the oldest repository of human knowledge and describes 67 plants. The Rigveda calls the plant the **"God for Gods"** seemingly giving him precedence above[2]. Indra and the other Gods. In the Vedas, the drink, and the plant refer to the same entity. Drinking *Soma* produces immortality (*Amrita*). Amrita is phonetically and conceptually very similar to the Greek ambrosia; both are what the gods drink, and what made them deities. Indra and Agni are portrayed as consuming Soma in copious quantities. The consumption of Soma by human beings is well attested in Vedic ritual. The ritual of Somayajna is still held with unbroken continuity in the South India. The *Somalatha* (Sanskrit: Soma creeper) which is procured in small quantities from the Himalayan region is used to prepare Soma rasam or Soma juice. It is also used in these areas in Ayurveda and Siddha medicine streams since time immemorial. The herb which is used is *Sarcostemma Acidum*. After Rigveda, Ayurveda is one of the oldest medical systems in the world. Ayurveda started on the slopes of the Himalayas some five thousand years ago. The Himalayas are the richest source of important medicinal plants used in Ayurveda. The unique holistic health concepts of Ayurveda together with its rich pharmacopoeia continue to have universal application and relevance.

The world health organization survey suggests that 70-80% of world populations rely on medicines that are obtained from plant sources in their primary healthcare[3]. The increase in such popularity of herbal medicines also brought the attention towards the safer use of such herbal medicines which are used frequently such as aphrodisiacs[4].

1.1 Aphrodisiacs

Sexual stimulants are still often called "aphrodisiacs" (after Aphrodite, the Greek goddess of love). Aphrodisiacs are the agents which are used extensively by the human beings seeking to

improve their sexual life and help in erectile dysfunctions (ED) sometimes called 'impotence'. Sexual desire is controlled and regulated by the central nervous system which integrates tactile, olfactory, specific auditory and mental stimuli. The aphrodisiac drugs act by altering the level of specific neurotransmitters or specific sex hormones into the body[5]. Most of the aphrodisiacs agent acts by altering the testosterone and progesterone concentration in the body.

1.1.1 Mechanism involved in Aphrodisiac potentials

On sexual stimulation (visual (or) otherwise the famines of the axons of parasympathetic nerves release nitric oxide (NO) gas. The gas diffuses into smooth muscle cells that line those arteries of the corpus carvenosum (spongy erectile tissue) and activates the enzyme guanylate cyclase (GC). The later converts the nucleotide guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cgmp). The cgmp in turn causes the smooth muscle cells around the penis to relax, leading to dilation and increased flux of blood into the penile tissue. This blood is essentially trapped in the penis and results in an erection. The erection ceases after a while because cgmp is hydrolyzed by phosphodiesterase type-5 enzyme (PDE-5) into inactive GMP. (The PDE-5 enzyme resides in the penile tissues). Aphrodisiac potentials inhibit the hydrolyzing action of PDE-5 with the result that active cgmp can accumulate. 'Undisturbed' and prolong the erection through increased blood flow[6,7].

1.2 Male sexual problems

Male sexual problems include libido, erection, ejaculation and orgasm. These sexual problems generally arises Male sexual response cycle is called normal if all the steps are timely and sequentially if any one of the following is not in sequence or delayed than it leads sexual dysfunction in humans. Main causes which are responsible for sexual problems include smoking, obesity, testosterone deficiency, depression, anxiety, alcoholism, and antidepressants and blood pressure medications. Libido refers to sexual need of individuals and it very person to person. Erection is enlarged condition of male reproductive organ. Ejaculation is ejection of semen during sexual intercourse, and orgasm represents intense pleasure condition during the climax of sexual response. One of the measure sexual problems arising in the modern time is due to erectile dysfunction which is also associated with depression, endocrine, neurologic, vascular, and systemic disorder[8].

1.3 Erectile dysfunction and risk factors

ED is a broad category that includes inability to achieve erection, or the ability to achieve only brief erections[9]. Besides of ED there are various sexual dysfunctions like Disorders of ejaculations, disorders of orgasm, and failure of detumescence. By the several years clinical and epidemiological studies it has been proved that several risk factors are associated with ED. These risk factors include smoking, age, obesity, and diabetes and also various stress conditions. An important point to notice here is that these risk factors are the same as the risk factors of cardiovascular disease. So even ED is not a life threatening disease, it may represent as an early sign of cardiovascular disease[10].

Synthetic drugs like Sildenafil citrate, Tadalafil citrate, Vardenafil, Tadalafil, Alprostadil, Papaverin are used for ED but these drugs also have fatal side effects like sudden hypotension, hypersensitivity reaction, abnormal vision, infertility, suicidal tendencies, mental disorders and tremors[11]. The use of synthetic aphrodisiacs results in the dilation of blood vessels in other parts of the body causing headache and fainting. Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses[12]**.**

Thus, there is growing need to look for aphrodisiacs more of natural plant or herbal origin as opposed to synthetic compounds which are known to cause severe unwanted side effects. Some of the most ancient plant-based aphrodisiacs, such as ginseng and yohimbine, are still as popular today as in ancient times. Unlike the old-time aphrodisiacs, which were meant only to increase sex drive and/or sexual pleasure, modern stimulants including Viagra, may rightly be called medications, since their purpose is to correct problems that make sex difficult or impossible. Besides of the fact that several plant sources contain aphrodisiac ingredients (phytochemicals) which can be beneficial as an immune modulator, sex stimulant and also as medication in erectile dysfunctions, there is very low range of research work carried out in this field.

According to traditional ayurvadic book herbal plants *Kigelia africana* and *Cannabis indica* Claimed to have good aphrodisiac potential but the actual action is not yet proved by scientific methods.

1.4 Plant profile

Image- captured from the garden of FRI, Dehradun

1.4.1 *Kigelia africana* (Lam.) Benth.

1.4.2 *Scientific classification*

Botanical Name: ***Kigelia africana* (Lam.) Benth.**

Family : **Bignoniaceae**

1.4.3 Vernacular names:

- Common name : Sausage tree(Eng.); worsboom (Afr.)
- Hindi : Balam khira (बkलम खीरा), Jhar fanoos (झाड़ फ़ानूस)
- Kannada : Aanethoradu Kaayi, Mara Sowthae
- Telugu : Enuga thondamu, Kijili.

1.4.4 Botanical description

The short, squat trunk has light brown, sometimes flaky bark and supports a dense rounded to spreading crown (18 m high, 20 m wide) of leathery, slightly glossy foliage (deciduous). The huge, grey-brown fruits, 800 x 120 mm. Hang from long stalks, from December to June and weigh anything up to 9 kg.

1.4.5 Occurrence and distribution

The tree is found on riverbanks, along streams and on floodplains, also in open woodland, from Kwazulu-Natal to Tanzania. The plant is widely distributed in the south, central and West Africa[14]. Also found in south Asia (India, Pakistan, Bangladesh, Srilanka, etc.

1.4.6 Ecology

Kigelia africana grows along watercourses, in riverine fringes, alluvial and open woodland, high rainfall savanna, shrub land and in rain forest. It occurs on loamy red clay soils, sometimes rocky, damp or peaty, from sea level up to zoom altitude[15].

1.4.7 Traditional and medicinal uses

In the African folk medicine K. Africana is used against dysentery, venereal diseases and as a anti-inflamatory,anti-microbial and anti-skin-aging effects and as topical application on wounds and abscesses. In Togo the stem bark is the component of a prescription against cancer:In order to enlarge the penis young males enrub the sap of the fruit into cuts of the penis skin. Young females do the same with the flesh of ripe fruits for enlarging their bosom**[17]**.

1.4.8 Part used

The fruits of *K. Africana* will be used.

1.4.9 Chemical constituent

The **roots**, the **wood** and the **leaves** have been investigated chemically. They contain naphthoquinones, dihydroisocoumarines, flavonoids and aldehydic iridoids. Among the naphthoquinones kigelinole, isokigelinole, pinnatal and isopinnatal were isolated .From the **roo**t and its **bark** Kigelin is the main component of the plant, The **root bark** and **stem bark** from plants afforded three naphthoquinones:

- Kigelinol
- Isokigelinol
- Isopinnatal

The **fruit** from *K. africana* contains **verminoside** ($C_{24}H_{28}O_{13}$), an **iridoid** as a major constituent, among a series of polyphenols such as verbascoside[17].

Pharmacological activities of different phytoconstituents of *Kigelia africana* are Anticancer, Mollucidal, Syphlis and Gonorrhea, Antidiarrhoeal, Antiulcer, Antifungal, Antimalarial, Antiinflamatory/analgesic, Antibacterial, Antiurolithic and Antihyperlipidemic[17]

1.5 *Cannabis indica*

Cannabis is a genus of flowering plant that includes three species - *sativa*, *indica*, and *ruderalis*. The plant is indigenous to central Asia and the Indian subcontinent. Cannabis has long been used for hemp fibre, for hemp oils, for medicinal purposes, and as a recreational drug[18,19].

Imagehttps://en.wikipedia.org/wiki/Cannabis_indica#/media/File:Indica_leaf.jpg

1.5.1 Scientific classification

Family: Cannabaceae

Genus: *Cannabis*

Species**:** *Cannabis indica* Lam[20].

1.5.2 Vernacular names

English : Hemp

Hindi : Bhang, Ganja, Joot

1.5.3 Part used

The leaves of Cannabis indica will be used

1.5.4 Botanical description

Cannabis is an annual, dioecious, flowering herb. The leaves are palmately compound or digitate, with serrate leaflets. The first pair of leaves usually have a single leaflet, the number gradually increasing up to a maximum of about thirteen leaflets per leaf (usually seven or nine), depending on variety and growing conditions. At the top of a flowering plant, this number again diminishes to a single leaflet per leaf. The lower leaf pairs usually occur in an opposite leaf arrangement and the upper leaf pairs in an alternate arrangement on the main stem of a mature plant[21].

1.5.5 Chemical constituents

Cannabis plants produce a group of chemicals called cannabinoids, which produce mental and physical effects when consumed. Cannabinoids, terpenoids, and other compounds are secreted by glandular trichomes that occur most abundantly on the floral calyxes and bracts of female plants. As a drug it usually comes in the form of dried flower buds (marijuana), resin (hashish), or various extracts collectively known as hashish oil[22].

1.5.6 Traditional and medicinal uses

Cannabis is a popular recreational drug around the world, only behind alcohol, caffeine and tobacco[23]. The psychoactive effects of *cannabis* are known to have a biphasic nature. Primary psychoactive effects include a state of relaxation, and to a lesser degree, euphoria from its main psychoactive compound, tetrahydrocannabinol. Secondary psychoactive effects, such as a facility for philosophical thinking, introspection and metacognition have been reported amongst cases of anxiety and paranoia[24]. Medical cannabis (or medical marijuana) refers to the use of cannabis and its constituent cannabinoids, to treat disease or improve symptoms. Cannabis is used to reduce nausea and vomiting during chemotherapy, to improve appetite in people with HIV/AIDS, and to treat chronic pain and muscle spasms[25,26]. Short-term use increases both minor and major adverse effects,Common side effects include dizziness, feeling tired, vomiting, and hallucinations[26]. Long-term effects of cannabis are not clear. Concerns including memory and cognition problems, risk of addiction, schizophrenia in young people, and the risk of children taking it by accident. Cannabinoids are under preliminary research for their potential to affect strokeor children's epilepsy[27,28].

2. Aim and Objectives

Despite progress made in modern medical science, the overall success rate has remained modest and unsatisfactory. Remission of limited duration and reappearance of symptoms are frequent as a result of a variety of deficiencies associated with present day drugs. Typical deficiencies associated with modern allopathic therapy include excessive systemic toxicity, Lack of cell specificity, due to the lack of the cell specificity drug application will be excessively wasteful, and healthy tissues will be exposed to toxic side effects. Because of these disadvantages associated with the modern day therapy there is a greater interest is increasing towards herbal drugs obtained from various plant sources. The current research work includes identification, extraction, isolation; characterization and pharmacological evaluation of aphrodisiac potential of *Kigelia africana* and *Cannabis indica a*re intended to look for safe and powerful aphrodisiac.

1. To collect Plant Material for identification and authentification.
2. To perform successive extraction of plant material.
3. To perform preliminary phytochemical screening of the extracts for the detection of various phytoconstituents (Alkaloids, steroids, tannins, flavonoids, saponins, carbohydrates, fatty acids etc).
4. To perform the pharmacological activity (aphrodisiac activities) of crude drug extracts by employing on wistar albino rats using different aphrodisiac rat models.
5. To perform pharmacological activity of most active extract and isolating molecules responsible for the activity.

3. Review of literature

- **Chauhan *et al.* 2009** worked on the roots of *Asparagus racemosus, Chlorophytum borivilianum,* and rhizomes of *Curculigo orchioides* for their aphrodisiac and immunostimulatory potential. The aphrodisiac activity of the different extracts of *A. Racemosus, C. Borivilianum,* and rhizomes of *C. Orchioides* were studied for sexual behaviour effects in male albino rats and compared with the control group. Administration of the dose 200 mg/kg body weight of the aqueous extract of the herbal plants showed a significant variation in the sexual behaviour of the animals and it was reflected by the reduction of mount latency, ejaculation latency, post ejaculation latency, intromission latency, and increase in mount frequency. Penile erection is also increased. The followings effects of the extracts are similar to the effects produced by the testosterone like. So the present study supports the fact that herbal drugs are useful source of aphrodisiac agents which also acts as immunomodulator**[31]**.

- The study by **Ratnasooriya *et al.* 2008** was based on Sri Lankan traditional medicine black tea brew of *Camellia sinensis* which is claimed to have male sexual stimulant activity. Different doses of black tea brew extract (167 and 501 mg/ml) or water was successfully given to separate groups of rats (n= 9 per group) and after three hours the sexual behaviour was monitored using receptive females. The result showed prolongation of latency of ejaculation, shortening of mount and intromission latencies and elevation of serum testosterone level. The overall result showed that black tea brews possess marked aphrodisiac activity. Toxicity studies showed that BTB is non toxic in terms of liver and renal toxicity**[32]**.

- **Mbatchou *et al.* 2012** evaluated the aphrodisiac activity of the *Anacardium occidentale* L. Phytochemical analysis of the seed-oil revealed the presence of saponins, alkaloids, flavonoids, steroids and terpenoids, while tannins were absent. The seed-oil was later tested on male albino rats for sexual behavior which resulted to significant increase in mount and

intromission frequencies, and decrease in mount latency. The seed shell oil of *Anacardium occidentale L.* Was tested on both male and female albino rats for toxicity which demonstrated mortalities at various doses (0.10, 0.60 and 1.10 ml). Results of this study revealed that the seeds and seed shells of *Anacardium occidentale L.* Have pharmacological and toxicological attributes. Thus, the seed oil of this plant should be used to manage impotency in male humans**[33]**.

- **Wani *et al.* 2011** studied the activity of the plant extract of *Asparagus racemosus***.** Based on preliminary reports, there is a lot of interest in using the roots of this plant for treating sexual disorders. In this study, the hydro-alcoholic and aqueous extracts of the roots of *Asparagus racemosus* were subjected to preliminary phytochemical screening which showed the presence of saponins, carbohydrates, glycosides and mucilages. The total extracts were tested for their aphrodisiac activity in experimental rats. The hydro-alcoholic extract of *Asparagus racemosus* root at higher concentration (400 mg/kg body weight) showed significant aphrodisiac activity on male wistar albino rats as evidenced by an increase in number of mounts and mating performance. This study suggested that the hydroalcoholic extract at lower dose (200 mg/kg body weight) and aqueous extract showed significant aphrodisiac activity**[34]**.

- **Gauthaman *et al.* 2002** from a long period of time *Tribulas terrestris* has being used as traditional Chinese and Indian systems of medicines because of its aphrodisiac activity. Sexual behaviour was studied on both normal and castrated rats. Adult Sprague-Dawley rats were divided in five groups of 8 each. One group is treated by distilled water (normal and castrated), one by testosterone (normal and castrated, 10 mg/kg body weight, subcutaneously, bi- weekly), *Tribulas terrestris* treated (castrated, 5mg/kg body weight, orally once daily) compared to the intact group. There was a mild to moderate improvement of the sexual behaviour parameters. After the entire study it is concluded that extract is having aphrodisiac activity probably due to androgen increasing property of *Tribulas terrestris***[35]**.

- **Singh *et al.* 2011** collected and studied the reviews of plants identified from various ethno botanical surveys and folklore survey. The complete study was based upon the aphrodisiac potential of the various medicinal plants obtained from various regions. The conclusion of the study was that the most of the natural plant sources are rich in aphrodisiac potential to treat the various types of body ailments. Because of the increasing demand of herbal drugs the plants should be subjected to animal and human studies to determine their effectiveness. The review contains the total record of the plants family, part used for aphrodisiac activity and the references from where this information is collected**[36]**.

4. Methodology

4.1 Sexual behavior study

Sexual behavior studies were monitored in a separate room for 30 min. Following the administration of standard drug & extracts and were given 20 minutes adaptation period, after which a primed female was introduced into the study cage. On 0, $7^{th,}$ 14^{th}, $21^{st,}$ and 28^{th} days sexual behaviors study were monitored. Experiment performed in the same environment during the dark phase of the cycle in large cage **(e.g. 60x60x60cm)** with a floor that is similar to the home cages. The following male sexual behavior patterns were recorded, including;

(a). Mount frequency (MF): number of mounts in series, or number of mounts in a given period of time. (2 hours)

(b). Intromission frequency (IF): number of intromissions observed in 2 hours.

(c). Mount latency: the time interval between the introductions of the female to the first mount by the male.

(d). Intromission latency: the interval from the time of introduction of the female to the first intromission by the male.

(f). Ejaculatory latency: the time interval between the first intromission and ejaculation.

(g). Ejaculation frequency: the number of ejaculation in a series.

(h). Post ejaculatory mount latency: time from ejaculation to next mount.

(i). Post ejaculatory interval: time from ejaculation until next intromission.

(j). Copulatory rate: the number of mounts plus number of intromissions divided by the time from the first mount until ejaculation[29].

4.2 Attraction towards female and determination of hesitation time

A female rat will be placed in a cage which has a wooden barrier of 15 cm separating male & female compartments which could be passed by a motivated male rat. The hesitation time were recorded as the time (in sec) required by the male rat before making an attempt to cross the barrier. In the same way, a scoring for attraction towards female was recorded by a score between 0-5 during an observation period of 15 min. A complete cross of the partition by the male rat each time will be considered as a score of 5 while an attempt to climb will be considered a score of 2 & disinterest to climb will be rated as 0. The readings should be recorded on 0, 7, 14, 21 and 28^{th} days of treatment. This test is useful in determining the willingness of a male rat to cross an aversive or obstructive position, thus indicating the intent of sexual attraction. Male rats of the entire group will be considered for experimentation & their scores for attraction as well as hesitation time will be recorded[29].

4.3 Partner preference test

Partner preference test, is used to evaluate sexual motivation in a no contact condition. The apparatus consist of an open field arena (100cmx50cmx40cm high) with two round cages made of wire meshing (16cm diameter and 40 cm high) diagonally positioned at the opposite corners of the arena. We will use two stimulus animals: a male in one cage and a receptive female in the other cage: the sexual incentive area near to the female and the social incentive area near to male. The transmissions of visual, olfactory and auditory cues will be allowed while mating will be avoided. Experimental males will be individually place in the centre of arena for a 5 min. Adaptation period at the presence of the stimulus animals and thereafter tested for 10 min. The number of visits to the male and the female rats as well as the time spent near each stimulus animal will be recorded. The measure of sexual motivation is expressed by a preference score, i.e. The ratio between the times spent in sexual incentive area and the total time spent in two incentive areas. The readings will be recorded on 0, 7, 14, 21 and 28^{th} days of treatment[30].

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